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Selective opening of the blood-tumor barrier by intracarotid infusion of leukotriene C_4

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✓ Intracarotid infusions of leukotriene C_4 (LTC_4) were used to selectively open the blood-tumor barrier in rats with RG-2 gliomas. Blood-brain and blood-tumor permeability was determined by quantitative autoradiography using ^{14}C aminoisobutyric acid. Leukotriene C_4 (4 μg total dose) infused into the carotid artery ipsilateral to the tumor increased twofold the unidirectional transfer constant for permeability within the tumor while no effect on permeability was seen in normal brain. No gamma glutamyl transpeptidase (γ-GTP) activity was seen in tumor capillaries in contrast to high γ-GTP in normal brain capillaries. These findings suggest that normal brain capillaries may resist the vasogenic effects of LTC_4, while LTC_4 will increase permeability in tumor capillaries. This could relate to the ability of γ-GTP to act as an enzymatic barrier and inactivate leukotrienes in normal brain capillaries. Intracarotid LTC_4 infusion may be a useful tool to selectively open the blood-tumor barrier for delivery of antineoplastic compounds.

KEY WORDS · brain neoplasm · blood-brain barrier · glioma · leukotriene · gamma glutamyl transpeptidase · rat

CAPILLARIES within primary brain tumors contain a blood-tumor barrier which impairs delivery of many antitumor moieties to tumor cells within the brain.[8,9,12] This inability to deliver antineoplastic compounds across the blood-tumor barrier has lead some workers to increase their focus on strategies to open this barrier.[10,12,14,16,20] One approach has utilized intracarotid infusions of mannitol to osmotically disrupt the tight junctions of brain endothelial cells in patients with brain tumors.[12,14,15,20] Neuwelt, *et al.*,[13] have shown that osmotic barrier disruption can nonspecifically increase the delivery of chemotherapeutic compounds and monoclonal antibodies to the brain. Osmotic disruption of the blood-brain barrier (BBB), however, results in only a modest increase in drug levels within the actual tumor compared to the large increase in drug levels in normal brain.[11] In effect, therefore, this technique increases the exposure of the normal brain parenchyma to the deleterious effects of the chemotherapeutic agents,[20] while suboptimal drug levels may actually reach the tumor cells. In this paper, we describe an experimental method that circumvents this problem. This technique selectively opens the barrier within the tumor but leaves the BBB intact.

In previous studies, we demonstrated a significant correlation between tissue levels of leukotriene C_4 (LTC_4) measured in brain tumors and the amount of vasogenic edema surrounding these tumors in man.[5] This, combined with our observation that brain capillaries have high affinity binding sites for LTC_4,[2] led us to speculate that LTC_4 might modulate capillary permeability in brain tumors.[3] Here we report on the ability of intracarotid infusions of LTC_4 to selectively open the tumor barrier in an experimental glial tumor, and we discuss both the biological and clinical implications of this observation.

Materials and Methods

Leukotriene C_4* was obtained at a concentration of 0.1 mg/ml in a 1:1 phosphate-buffered saline (PBS)-ethanol solution. Aliquots of LTC_4 were diluted to experimental concentrations in PBS and the final ethanol concentration was adjusted to 2.5%. Vials containing LTC_4 were sealed under nitrogen and stored at −80°C. Radiolabeled LTC_4 (14,15−^3H(N)−LTC_4) (38.4 Ci/mmol) and alpha-(1−^{14}C)-aminoisobutyric acid

*LTC_4 obtained from Cayman Chemicals, Ann Arbor, Michigan.

(AIB) (50.0 mCi/mmol) were used for quantitative study.†

Intracarotid Infusion Experiments

The RG-2 glioma cell line was maintained in a monolayer culture in Dulbecco's minimum essential medium with 10% calf serum. Female Wistar rats, each weighing 100 to 150 gm, were anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (0.8 mg/kg). Glial tumors were implanted into the left hemisphere by intracerebral injections of 5×10^4 RG-2 glioma cells in 10 μl of serum-free F10 medium. Thirteen days after tumor implantation, the rats were again anesthetized and a polyethylene (PE-10) catheter was inserted retrograde through the external carotid artery to the common carotid artery bifurcation ipsilateral to the tumor. The external carotid artery was then ligated. Both femoral arteries and one femoral vein were also cannulated.

Body temperature was maintained at 37°C and arterial blood gas levels, blood pressure, and hematocrit were monitored. Animals with abnormal physiological parameters were eliminated from this study. Leukotriene C_4 (0.8 ml) at a concentration of 5 μg/ml or 0.8 ml of vehicle (2.5% ethanol in PBS, pH 7.1) was infused into the left carotid artery by means of a constant infusion pump at a rate of 53.3 μl/min for 15 minutes. Five minutes after the start of the intracarotid infusion, 100 μCi/kg of ^{14}C-AIB was injected as an intravenous bolus. A peristaltic withdrawal pump was used to withdraw femoral arterial blood at a constant rate of 0.083 ml/min immediately after injection of ^{14}C-AIB for determination of serum radioactivity. Fifteen minutes after the start of intracarotid infusions, the animals were killed by decapitation and the brains were rapidly removed and frozen.

Autoradiography

The frozen brains were mounted onto pedestals with M-1 embedding matrix, and 20 μm coronal sections were cut with a cryotome. The sections were thaw-mounted onto cover slips, and autoradiograms were generated by coexposing the sections on Kodak XAR-5 film with tissue-calibrated ^{14}C standards‡ for 2 weeks. After exposure, the sections were stained with thionine for correlation of areas of histologically verified tumor autoradiograms. Quantitative analysis was performed using a computer-assisted digital image analyzer at the Laboratory of Neuro-Imaging, University of California School of Medicine, Los Angeles.[19] A unidirectional blood-to-brain transfer constant, K_i, was calculated using the method described by Blasberg, *et al.*,[6] and was expressed in μl/gm/min.

In Vitro LTC₄ Binding

To determine specific binding of ^{3}H-LTC_4 to rat brain tissue and RG-2 tumors *in vitro*, brains from rats with and without RG-2 tumors were removed, frozen, and cut in 20-μm coronal sections using a cryotome. The sections were separated in an alternate fashion to obtain adjacent sections to subtract nonspecific from total binding images. Specific ^{3}H-LTC_4 binding images were obtained by subtracting nonspecific binding images from total binding images pixel by pixel, using a digital image analyzer. The sections were placed in a dessicator and kept in a freezer overnight. The sections were washed twice in ice-cold binding buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM L-serine, 10 mM sodium borate, pH 7.4) for 4 minutes and cool-air dried; they were then immersed into binding buffer containing 2.5 nM ^{3}H-LTC_4 for 60 minutes at 0° to 4°C with or without 10 μM LTC_4, and were again washed in binding buffer and cool-air dried. Autoradiograms were generated by exposing the sections of ^{3}H Hyperfilm for 2 weeks with tissue-calibrated ^{3}H standards.§

Gamma Gluramyl Transpeptidase Histochemistry

Rat brains with gliomas were fixed in 70% ethyl alcohol and embedded in paraffin. Six-micron sections were cut, deparaffinized, acetone-fixed, and incubated at 37°C for 60 minutes in a gamma glutamyl transpeptidase (γ-GTP) reaction mixture of 0.5 mM γ-glutamyl-4-methoxy-2-naphthylamide, 15mM glycylglycine, and 0.05% fast blue BB in 25 mM phosphate buffer (pH 7.2) containing 0.25% dimethylsulfoxide. Sections were then washed with distilled water, rinsed with 0.9% saline for 2 minutes, and placed in 0.1 M $CuSO_4$ for 2 minutes. Counterstaining of sections with hematoxylin was performed for 1 minute.

Data Analysis

The K_i values for the experiments were calculated by measuring regions of interest in three consecutive sections. Analysis of variance and Student's t-test were applied to the mean values from separate experiments.

Results

Intracarotid infusion of LTC_4 resulted in a twofold increase in brain-tumor barrier permeability (K_i) within tumors which were 3 mm in diameter or smaller, when compared to vehicle alone (mean ± standard error of the mean for nine rats in each group: 45.58 ± 6.61 vs. 22.33 ± 2.35 μl/gm/min, $p < 0.003$). There was no significant change in BBB permeability of the ipsilateral cortex 2.97 ± 1.37 vs. 2.39 ± 1.09 μl/gm/min), contralateral cortex (2.87 ± 1.36 vs. 2.80 ± 1.27 μl/gm/min), or contralateral corpus callosum (2.30 ± 0.92 vs. 0.89 ± 0.53 μl/gm/min). A modest increase in permeability

† Radiolabeled LTC_4 and AIB obtained from New England Nuclear, Boston, Massachusetts.

‡ ^{14}C standards obtained from Amersham Corp., Arlington Heights, Illinois.

§ ^{3}H Hyperfilm and ^{3}H standards obtained from Amersham Corp., Arlington Heights, Illinois.

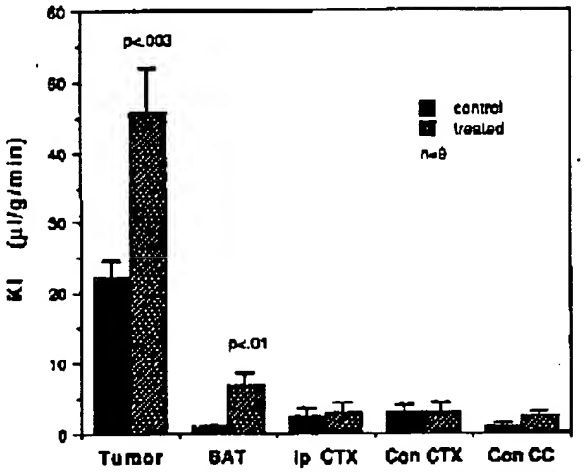

FIG. 1. Effect of intracarotid infusion of either leukotriene C_4 (nine rats) or vehicle (nine rats) on blood-brain and blood-tumor barrier permeability. Values for the unidirectional transfer constant, K_i, are shown as the mean ± standard error of the mean. BAT = brain adjacent to tumor; Ip CTX = ipsilateral cortex; Con CTX = contralateral cortex; Con CC = contralateral corpus callosum.

was seen in the basal ganglia immediately adjacent to the tumors (6.63 ± 2.05 vs. 0.88 ± 0.42 µl/gm/min, $p < 0.01$) although no tumor infiltration was seen in this area (Fig. 1). In tumors larger than 3 mm, K_i values ranged between 85 and 95 µl/gm/min and were significantly not increased by LTC_4 infusions.

The ^{3}H-LTC_4 binding experiments revealed some specific binding in normal brain (66.15 ± 6.19 fmol/mg tissue at 2.5 nM) but little specific binding in tumors (25.04 ± 3.34 fmol/mg tissue). There was no γ-GTP activity in tumor capillaries in contrast to normal brain (Fig. 2). Preliminary results suggested a slight decrease in γ-GTP levels in some areas of normal tissue surrounding tumors. The LTC_4 infusions did not alter the physiological parameters.

Discussion

Leukotrienes are pharmacologically active compounds that promote vascular permeability.[3,17,18,20] This study demonstrates that the blood-tumor barrier in an experimental glial tumor can be selectively opened by intracarotid infusion of LTC_4 without increasing permeability in the normal cortex or white matter. Clinically, intracarotid LTC_4 infusions could therefore be an extremely useful tool to increase delivery of antitumor moieties within tumors without increasing the concentrations of potentially toxic compounds in normal brain. The mechanism(s) for the selective effect of LTC_4 on capillary permeability in tumors compared to normal brain remains unclear. One possibility could reside in the ability of normal brain capillaries to resist the vasogenic effects of LTC_4. Leukotriene C_4 will markedly increase permeability in a variety of (noncerebral) systemic capillary beds.[17,18,21] In prior studies, we have demonstrated that intraparenchymal injections into the brain of high doses of leukotrienes would increase BBB permeability.[6] However, lower physiological doses of LTC_4 do not dramatically increase permeability in normal brain tissue (unpublished data). Brain capillaries, unlike systemic capillaries, are rich in γ-GTP,[7] an enzyme that metabolizes the peptidoleukotrienes LTC_4 and LTE_4 to LTD_4 and LTF_4, respectively.[1] High levels of γ-GTP are not present in the capillaries of experimental tumors. Theoretically, at physiological concentrations, LTC_4 could be inactivated by γ-GTP in normal capillaries, while tumor capillaries (which lack γ-GTP) are susceptible to the vasogenic effects of LTC_4.

In man, levels of LTC_4 are significantly increased in tumors compared to normal tissue and there is a significant correlation between LTC_4 levels and the amount of vasogenic edema surrounding these tumors.[5] If tumor capillaries are also more sensitive to the effects of LTC_4, as the present study suggests, this compound could play an important role in the formation of vasogenic edema surrounding brain tumors. Leukotrienes may also play a role in vasogenic brain edema formation in other pathological conditions where the resistance of normal brain capillaries to the effects of LTC_4 may be impaired.

Telologically, the ability of normal brain capillaries to resist LTC_4-induced permeability and the inability of abnormal capillaries to do so could be particularly advantageous. Leukocytes, rich in the 5-lipoxygenase enzyme and a major source of leukotrienes, could effectively open capillaries in abnormal areas and enter the brain parenchyma where they are needed while normal areas could resist their effects.

Although high-affinity binding sites for LTC_4 on isolated brain capillaries have been demonstrated,[5] it is not clear whether these binding sites are functional receptors for LTC_4 or whether the receptor is located in a γ-GTP-LTC_4 receptor complex. *In vitro* binding experiments in this study showed a relative paucity of high-affinity binding sites within tumors. This observation raises several possibilities, including 1) that the binding site is a γ-GTP-LTC_4 receptor complex, which the tumors may lack, or 2) that the mechanism of increased permeability by LTC_4 may not be receptor-mediated. These preliminary binding results do not, however, exclude the possibility of a functional LTC_4 receptor on tumor capillaries since optimal binding conditions may not have been applied.

The increased permeability seen in the basal ganglia adjacent to tumor suggest that capillaries in these areas may also be abnormal. This could relate to tumor angiogenesis or other secondary effects induced by the tumor which may also make capillaries in these areas more susceptible to the effects of LTC_4. Of interest is the preliminary observation that γ-GTP levels appear

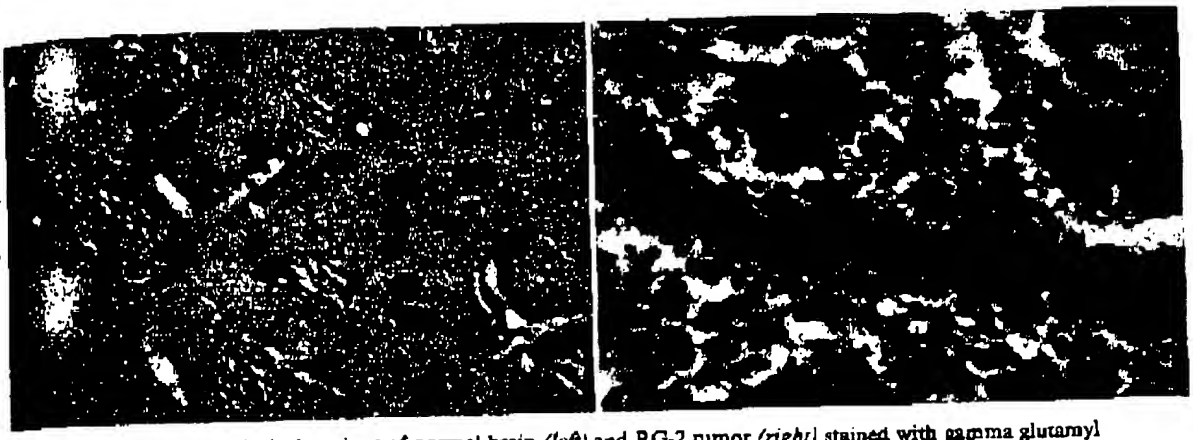

FIG. 2. Histological sections of normal brain *(left)* and RG-2 tumor *(right)* stained with gamma glutamyl transpeptidase (γ-GTP). Little staining is seen in the tumor compared to γ-GTP staining in capillaries within normal brain. *Arrows* show capillaries in normal brain stained with γ-GTP and a tumor capillary without activity. × 200.

to be decreased in some areas of normal brain surrounding these tumors.

To our knowledge, the observation that the normal BBB may have an enzymatic barrier to selectively resist increased permeability induced by biologically active compounds compared to systemic capillaries is unique. These findings add to our understanding of the biology of BBB in both normal and pathological conditions. The fact that intracarotid infusions of LTC_4 will selectively open the tumor barrier but not the BBB also has major clinical implications. Since intracarotid infusions of LTC_4 do not appear to produce any overt toxicity, this agent could be used to selectively increase delivery of a variety of antitumor moieties to tumor cells within the brain without increasing the exposure of normal tissue to the deleterious effects of antineoplastic agents.

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